

DP Barcode: 361785

MRID No.: 47622101

**DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE OPPTS 850.1500**

1. **CHEMICAL:** Triticonazole **PC Code No.:** 125620

2. **TEST MATERIAL:** BAS 595 F (Triticonazol) **Purity:** 93.6%

3. **CITATION**

Authors: Zok, S.

Title: BAS 595 F (Triticonazol) – Life cycle Test on the Fathead Minnow (*Pimephales promelas*) in a Flow-Through System.

Study Completion Date: August 7, 2008

Laboratory: Experimental Toxicology and Ecology
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Laboratory Report ID: 2008/1028361

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4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: *Christie E. Padova*

Date: 04/06/09

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: *Teri S. Myers*

Date: 04/15/09

5. **APPROVED BY:** Kristina Garber, OPP/EFED/ERB4

Signature: *Kristina Garber*

Date: 06/08/09

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism: Fathead minnow (*Pimephales promelas*)

Age of Test Organism: F₀ embryos, ≤6 hours post-fertilization

Definitive Test Duration: 6 months

Study Method: Flow-through



Type of Concentrations: Time-weighted average (TWA)

8. **CONCLUSIONS:**

Results Synopsis:

NOAEC: 12 µg ai/L

LOAEC: 23 µg ai/L

Endpoint(s) Affected: F₂-generation growth (length and weight) at 8 weeks post-hatch

9. **ADEQUACY OF THE STUDY:**

A. **Classification:** SUPPLEMENTAL

B. **Rationale:** See guideline deviations below.

C. **Repairability:** not repairable

10. **GUIDELINE DEVIATIONS:**

1. The environmental conditions maintained for the source culture were not reported.
2. Pre-test mortality of the source culture was not reported.
3. Only 25 embryos per replicate were exposed (100 per level) for the F₁ generation, whereas a minimum of 50 per replicate are recommended (200 per level).
4. The F₁ exposure period was notably shorter than recommended. Reproduction during the F₁-generation exposure was monitored for a 15-day period beginning on day 104 (ca. 14 and 15 weeks post-hatch), and the F₁-generation exposure was terminated on day 131, approximately 18 weeks post-hatch. However, guidance recommends establishing the spawning pairs around 20 to 24 weeks post-hatch, and terminating the study when no spawning has been observed for 1 week (approximately 32 to 40 weeks post-hatch).
5. The pH (of up to 8.0) was slightly higher than recommended (up to 7.6) for both generations.
6. Excessive analytical variation (high:low ratios ≥ 1.5) was observed at all levels during the F₁-generation pre-spawning and F₂-generation exposure periods.

11. **SUBMISSION PURPOSE:** Action Code - 575

12. **MATERIALS AND METHODS:**

A. Biological System:

Guideline Criteria	Reported Information
Species: Prefer sheepshead minnow (<i>Cyprinodon variegatus</i>) or fathead minnow (<i>Pimephales promelas</i>).	Fathead minnow (<i>Pimephales promelas</i>)
Source and acclimation	Fish used for egg production were from Osage Catfisheries, Inc., Osage Beach, MO (received February 2007). Parent fish were kept in groups of 1 male plus 2 female, and the eggs were collected from 14 different parental groups. Environmental conditions and pre-test mortality of the source culture were not reported.
Age at beginning of test: Embryos 2 to 24 hours old	Embryos in the blastula stage (verified using a stereomicroscope), ≤ 6 hours old
Feeding: Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	Newly-hatched larvae were fed freshly-hatched live brine shrimp nauplii (<i>Artemia salina</i>) twice daily. Adults were fed commercial diet and larvae of <i>Artemia salina</i> twice daily, <i>ad libitum</i> . The food was increased with the size of the fish. Feeding was stopped <i>ca.</i> 24 hours prior to sacrifice.

Guideline Criteria	Reported Information
<p>Embryo Exposure (Four-Five Days): Embryos (≤ 24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups.</p> <p>A minimum of 50 embryos (≤ 24 hrs old) per replicate cup, 4 cups per treatment should be used.</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • Survival of embryos • Time required to hatch • Hatching success • Survival of fry for 4 weeks <p>Dead and fungused embryos should be counted and removed daily.</p>	<p><u>Embryo Exposure</u></p> <p>Embryos (≤ 6 hours old), obtained from 14 separate spawns were impartially distributed into exposure vessels.</p> <p>Each vessel contained 25 embryos, with four replicate vessels per treatment level (total of 100 embryos per treatment).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • % survival from start to hatch <p>Dead embryos were counted and removed daily.</p>
<p>Larval-Juvenile Exposure (From Hatch to 8 Weeks): After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed.</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly) • Total lengths (mm) of all fish at 4 and 8 weeks after hatching. 	<p><u>Larval-Juvenile Exposure</u></p> <p>On day 18 (12 days post-hatch), fish were transferred from the hatching chambers to larger exposure aquaria.</p> <p>On day 36 (30 days post-hatch), surviving larvae were impartially thinned to 15 per replicate (60 per level).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • % survival from hatch to swim up • % survival from swim-up to reduction • Total length (cm) of all fish at 36 (prior to reduction) and 68 days (30 and 62 days post-hatch, respectively) <p>The vessels were examined daily for dead larvae/fish, and during reduction, all fish were observed for malformations.</p>

Guideline Criteria	Reported Information
<p>Juvenile-Adult Exposure (From 8 wks post-hatch to the end of the spawning phase [32-40 wks]):</p> <p>At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates.</p> <p>The substrates are examined daily and embryos removed, counted, and recorded separately for each pair.</p> <p>For fathead minnow, adult exposure should be terminated when no spawning occurs for one week.</p> <p>For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.</p>	<p><u>Juvenile-Adult Exposure</u></p> <p>On day 68 (62 days post-hatch), three spawning tiles were placed in each aquarium.</p> <p>On day 95, eight spawning pairs (one male + one female) were isolated per level; the remaining fish were maintained as reserve animals. On day 104, egg number and fertility rate was monitored daily for exactly 15 days. At day 117 (~16 weeks post-hatch), all F₁ fish were sacrificed.</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • % survival from reduction to reproduction • % survival from reproduction to study termination of pairs (Day 131; ~18 weeks post-hatch) • Gender-specific total lengths (cm) and wet weights (g) at study termination (Day 131; ~18 weeks post-hatch) • % fertility • No. eggs/female/day • No. spawns/female/day
<p>Second Generation Embryo Exposure (4-5 days):</p> <p>50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation.</p> <p>Embryos not selected are discarded.</p>	<p><u>Embryo Exposure</u></p> <p>The F₂ exposures were initiated on days 116 and 117. Each vessel contained 25 embryos (originating from one specific egg clutch), with four replicate vessels per treatment level (total of 100 embryos per treatment).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • % survival from start to hatch

Guideline Criteria	Reported Information
<p>Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 wks): After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment).</p> <p>Each group of 2nd generation fish is terminated 8 wks after hatching.</p> <p>Fish are blotted, weighed, and measured before being discarded.</p>	<p><u>Larval-Juvenile Exposure</u> Newly-hatched larvae were not thinned. Each group of F₂-generation fish was terminated <i>ca.</i> 8 weeks after hatching (day 64, or 59 days post-hatch).</p> <p><u>Parameters measured:</u></p> <ul style="list-style-type: none"> • % survival from hatch to swim-up • % survival from swim-up to study termination on day 64 • Total length (cm) at days 35 and 61 • Wet weight (g) at day 61

Comments: Although the first exposed generation is typically referred to as the F₀-generation in full life-cycle toxicity tests, in this study, the (unexposed) source fish were referred to as the F₀ generation, the initial exposed organisms as the F₁ generation, and the offspring exposed organisms as the F₂ generation.

For the F₁-generation, the maximum loading in the reserve group determined at the end of the exposure period was 0.39 g/L/day in replicate A of the nominal 3 µg/L treatment group. The maximum loading rate in the pair groups was 0.23 g/L/day. For the F₂-generation, the maximum loading rate was 0.18 g/L/day at the end of the exposure period. Thus, the maximum loading rate in the study did not exceed 0.4 g/L/day.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: <u>Fathead Minnow</u> 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as CaCO ₃ and pH of 7.2 to 7.6.	1. Aerated non-chlorinated charcoal-filtered tap water from the municipal water works of the city of Frankenthal, mixed with deionized water prepared in the testing facility. 2. 0.98 to 1.04 mmol/L
Test Temperature: <u>Fathead</u> : 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.	Daily measurements: 24 to 26°C Continuous measurements: 23.2 to 25.9°C
Photoperiod: 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	16-hour light/8-hour dark <u>Intensity (near the water surface):</u> F ₁ - and F ₂ -gen. early-life stages: 255-515 Lux F ₁ -gen. juvenile-adult stages: 102-230 Lux F ₁ -gen. pair groups: 87-257 Lux F ₂ -gen. juvenile stage: 162-419 Lux
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤0.5. 3. One control should be used.	1. Continuous-flow diluter. 2. Five toxicant concentrations with a dilution factor of 0.5. 3. A dilution water (negative) control was used.

Guideline Criteria	Reported Information
<p>Toxicant Mixing:</p> <ol style="list-style-type: none"> 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked. 	<ol style="list-style-type: none"> 1. The diluter system incorporated a mixing chamber. 2. Yes 3. The flow rates were calibrated (maximum deviation <10%) prior to exposure and once weekly during exposure.
<p>Exposure System/Test Vessels: Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead).</p> <p>Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm.</p> <p>Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.</p>	<p>Egg, larvae, and juveniles (F₁ and F₂ generations) were exposed in 1.7-L cylindrical glass vessels with plastic gauze-covered outlets.</p> <p>On day 18 (F₁ and F₂ generations), fish were transferred to 30-L glass aquaria with a fill volume of 24 L.</p> <p>Spawning pairs were exposed in stainless steel aquaria (29 cm L x 21 cm W x 22 cm H), with a 9-L fill volume (15-cm H). The overflow was covered with stainless steel gauze. Spawning tiles were plastic pipes that had been halved.</p> <p>During exposure, aquaria were covered with transparent lids.</p>
<p>Embryo and Fry Chambers:</p> <p>120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.</p>	<p>See above.</p>

Guideline Criteria	Reported Information
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	≥ 5 volume turnovers/day (all life stages and exposure vessels)
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	To ensure DO remained $>60\%$ saturation, aeration was initiated on day 22 for the F ₁ generation; day 106 for the F ₁ generation pair groups; and day 145 for the F ₂ generation. DO was maintained at $\geq 72\%$ saturation (all life stages and exposure vessels)

Comments: During the exposure period, water conductivity measured in the water supply ranged from 257 to 270 μS , and the total organic carbon measured in the water supply ranged from 1.3 to 2.4 mg/L. The water acid capacity (K) was also determined in the water supply. At an acid concentration of 0.02 mol/L and pH of 4.3, the consumption was 2.12 to 2.22 mmol/L.

C. Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations: Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate.</p> <p>Toxicant conc. must be measured in one tank at each toxicant level every week.</p>	<p>Nominal: 0 (negative control), 3, 6, 12, 24, and 48 µg/L</p> <p><u>Mean-measured:</u> F₁ pre-spawning: <LOQ (control), 3.1, 6.1, 11.7, 24.6, and 48.5 µg ai/L F₁ spawning pairs: <LOQ (control), 2.7, 5.8, 11.0, 21.6, and 42.5 µg ai/L F₂ generation: <LOQ (control), 3.0, 6.0, 11.6, 22.5, and 47.7 µg ai/L</p> <p><u>Time-weighted averages (reviewer-calculated):</u> F₁ pre-spawning: <LOQ (control), 3.1, 6.1, 12.1, 24.9, and 48.9 µg ai/L F₁ spawning pairs: <LOQ (control), 2.8, 5.8, 11.0, 21.8, and 43.0 µg ai/L F₂ generation: <LOQ (control), 3.1, 6.1, 11.8, 23.0, and 49.2 µg ai/L</p> <p>Test water samples were collected from all levels at study initiation and at least once weekly thereafter from one alternating replicate per level. The analytical LOQ was 1 µg ai/L.</p>

Guideline Criteria	Reported Information
<p>Other Variables:</p> <ol style="list-style-type: none"> 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. <u>Freshwater</u>: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. <u>Natural seawater</u>: must maintain a constant salinity and not fluctuate more than 6‰ weekly; monthly pH range <0.8 pH units. 	<ol style="list-style-type: none"> 1. DO was measured every 3 to 4 days in all replicate aquaria for each level. 2. Temperature was measured once daily in one alternating replicate aquaria for each level. Temperature was also continuously monitored (and recorded every 30 minutes) in one control replicate. 3. pH was measured generally every 3 to 4 days in one alternating replicate aquaria for each level. <p>Hardness, conductivity, acid capacity, and total organic carbon (TOC) were also periodically determined at all levels throughout the study.</p>
<p>Solvents: Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</p>	<p>N/A (see comment below)</p>

Comments: On several occasions throughout the study, the test concentrations were measured in all replicates. Results were in good agreements with each other, demonstrating consistency in the udder distribution system.

The dilution water was saturated with test material using saturation columns. The columns were prepared in the following manner: *ca.* 10 g of test substance was dissolved in *ca.* 200 mL acetone, and the solution was poured on *ca.* 20 g of glass wool in a stainless steel pan. The acetone was allowed to completely evaporate off, and the glass wool was placed in a column and secured with a cellulose plug. Each column was washed with dilution water for ≥ 1 day prior to use, and two columns were used in parallel. Each week, one of the columns was exchanged so that the use period for each column was *ca.* 2 weeks, and the use period of the two columns was overlapping. The outflow of the columns was introduced into a tank with an overflow, from which it was further diluted for each test concentration.

Water samples were diluted with an acetonitrile:water mixture and acidified with formic acid prior to analysis using HPLC/MS. The limit of quantitation (LOQ) was 0.001 mg ai/L.

As OPPTS 850.1500 guidance is not specific regarding analytical variation, the reviewer referred to OPPTS 850.1000 "Special considerations for conducting aquatic laboratory studies". In this guidance, it is reported that the ratio of the highest concentration to the lowest concentration should not exceed 1.5:1. High:low ratios were ≥ 1.5 for all levels during the F₁-generation pre-spawning and F₂-generation exposure periods (refer to copy of TWA worksheet in Appendix II). All high-low ratios were < 1.5 for the F₁-generation spawning period.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Data Endpoints must include:</p> <ul style="list-style-type: none"> • survival of F₀ and F₁ embryos, time required to hatch, and hatching success; • survival and total length of F₀ fish at 4 and 8 weeks after hatching; • weights and lengths of F₁ fish at 8 weeks; • incidence of pathological or histological effects; and • observations of other effects or clinical signs. 	<p>Data endpoints included:</p> <ul style="list-style-type: none"> • survival of F₁ embryos, time to hatch, time to swim-up, and hatching success; • survival of F₁ fish at swim-up; • survival and length of F₁ fish at 4 and 8 weeks post-hatch; • length and weight of F₁ fish (gender-specific) at termination of pairs (Day 131); • reproduction of F₁ fish, including time to maturation, % fertility, eggs per female per day, and clutches per female per day; • survival of F₂ embryos, time to hatch, time to swim-up, and hatching success; • survival of F₂ fish at swim-up and 8 weeks post-hatch; • length of F₂ fish at 4 and 8 weeks post-hatch; • weight of F₂ fish at 8 weeks post-hatch • incidence of behavioral or physical abnormalities

Toxicity Observations F₁ Generation:

Survival: There were no treatment-related effects on F₁-generation survival observed, with no statistically-significant differences indicated for any level or interval. Survival was $\geq 75\%$ up through 30 days post-hatch (at which time the fry were reduced to 60 per level), and $\geq 95\%$ from 30 days post-hatch to the initiation of spawning groups (on Day 95) and from the initiation of spawning groups to study termination (Days 95 to 116/117).

Nominal Conc. ($\mu\text{g ai/L}$) ^(a)	Survival									
	At Hatch (Day 6) ^(b)		At End of Swim-up		At Reduction (Day 36) ^(c)		At Start of Reproduction (Day 68)		At Termination (Days 116/117)	
	No.	%	No.	%	No.	%	No.	%	No. ^(d)	%
Negative control	84	84	77	92	75	97	60	100	54/58	93
3	86	86	81	94	77	95	60	100	59/59	100
6	87	87	76	87	75	99	59	98	57/59	97
12	91	91	82	90	79	96	57	95	52/55	95
24	89	89	80	90	78	98	59	98	57/58	98
48	89	89	80	90	78	98	60	100	58/58	100

^(a) Reviewer-calculated TWA concentrations were <1 ($<\text{LOQ}$, control), 3.1, 6.1, 12, 25, and 49 $\mu\text{g ai/L}$, respectively, for the F₁ generation prior to pairing and <1 (LOQ , control), 2.8, 5.8, 11, 22, and 43 $\mu\text{g ai/L}$, respectively, during the spawning phase (refer to Appendix II for copy of Excel worksheet).

^(b) 100 initial embryos per level.

^(c) Fish were thinned at Day 36 (30 days post-hatch) to 15 per replicate (60 per level).

^(d) Fish that jumped out of the vessels were considered to be "not at risk".

Time to hatch, time to swim-up, and clinical signs of toxicity: No treatment-related effect on the time to hatch or time to swim-up was indicated. The hatch in all test groups occurred simultaneously with the control group, where all eggs were derived from the same egg pool. Hatching occurred from Days 2 through 6, and swim-up occurred from Days 4 to 7. In addition, no substance-related signs of toxicity were observed following hatch.

Growth: At 4 weeks post-hatch (Day 36), a slight statistically-significant reduction in body length was indicated at the 12 $\mu\text{g ai/L}$ level compared to the control. Since the deviation was slight, not observed at the subsequent interval, and not dose-dependent, it was not considered to be related to treatment. At 8 weeks post-hatch (Day 68), statistically-significant increases in body length were observed at the 3 ($p \leq 0.01$) and 6 $\mu\text{g ai/L}$ ($p \leq 0.05$) treatment levels compared to the control. However, as the differences were slight ($<10\%$), not dose-dependent, and represented improvements for this parameter, they were regarded as most likely incidental to

treatment. No statistically-significant differences in growth were observed in males or females at pair-group termination (Day 131).

Nominal Conc. ($\mu\text{g ai/L}$) ^(a)	Body Length, Mean \pm SD (cm)				Body Weight, Mean \pm SD (g)	
	Day 36 (30 days post-hatch)	Day 68 (62 days post-hatch)	Day 131 (Pair-group sacrifice)			
	Combined Sexes	Combined Sexes	Males	Females	Males	Females
Negative control	2.77 \pm 0.333	4.43 \pm 0.642	6.7 \pm 0.28	5.4 \pm 0.54	4.46 \pm 0.51	2.10 \pm 0.29
3	2.77 \pm 0.333	4.82 \pm 0.555**	6.9 \pm 0.38	5.7 \pm 0.44	4.65 \pm 1.04	2.33 \pm 0.56
6	2.68 \pm 0.359	4.71 \pm 0.565*	6.6 \pm 0.41	5.7 \pm 0.19	4.44 \pm 0.83	2.24 \pm 0.31
12	2.60 \pm 0.416**	4.61 \pm 0.512	6.9 \pm 0.17	5.7 \pm 0.34	4.37 \pm 0.35	2.32 \pm 0.92
24	2.68 \pm 0.337	4.63 \pm 0.548	6.7 \pm 0.27	5.6 \pm 0.24	4.22 \pm 0.54	2.10 \pm 0.41
48	2.73 \pm 0.295	4.66 \pm 0.482	6.7 \pm 0.34	5.5 \pm 0.23	4.43 \pm 0.75	2.02 \pm 0.27

^(a) Reviewer-calculated TWA concentrations were <1 ($<\text{LOQ}$, control), 3.1, 6.1, 12, 25, and 49 $\mu\text{g ai/L}$, respectively, for the F_1 generation prior to pairing and <1 (LOQ , control), 2.8, 5.8, 11, 22, and 43 $\mu\text{g ai/L}$, respectively, during the spawning phase (refer to Appendix II for copy of Excel worksheet).

* Statistically-significant difference from the control at $p \leq 0.05$.

** Statistically-significant difference from the control at $p \leq 0.01$.

Reproduction: The time to reproductive maturation was defined as the interval between Day 68 (when the spawning substrates were introduced into each tank) and the start of reproduction (when the first eggs were laid), and was evaluated qualitatively. The mean time to reproductive maturation was 75, 82, 83, 81, 79, and 86 days for the control, 3, 6, 12, 24, and 48 $\mu\text{g ai/L}$ levels, respectively. It was reported that the data may indicate a slight delay in the time to maturation in the highest treatment level, but that the variability of this parameter is not well known, and only a severe delay in maturation could be clearly attributed to the test substance.

Additional reproductive parameters assessed included the number of eggs per pair per day, the percentage of fertile eggs, and the number of clutches per day and pair. No statistically-significant differences from the control were observed for any endpoint.

Nominal Conc. ($\mu\text{g ai/L}$)	Mean Time to Maturation (Days) ^(b)	Eggs/day/pair	% Fertile eggs	Clutches/day/pair
Negative control	75	17.9	98.6	0.225
3	82	17.0	98.4	0.171
6	83	16.4	98.3	0.175
12	81	17.3	99.0	0.175
24	79	17.4	99.4	0.133
48	86	14.3	98.3	0.217

^(a) Reviewer-calculated TWA concentrations were <1 (<LOQ, control), 3.1, 6.1, 12, 25, and 49 $\mu\text{g ai/L}$, respectively, for the F₁ generation prior to pairing and <1 (LOQ, control), 2.8, 5.8, 11, 22, and 43 $\mu\text{g ai/L}$, respectively, during the spawning phase (refer to Appendix II for copy of Excel worksheet).

^(b) Data not statistically analyzed.

Toxicity Observations F₂ Generation:

Survival: There were no treatment-related effects on F₂-generation survival up through the end of the 8-week exposure, with no statistically-significant differences indicated. For all levels, survival was $\geq 93\%$ from study initiation to hatch, $\geq 91\%$ from hatch to the end of swim-up, and $\geq 90\%$ from the end of swim-up to study termination. Overall survival averaged 77% for the control group, 83, 85, 81, 84, and 84% for the 3, 6, 12, 24, and 48 $\mu\text{g ai/L}$ levels, respectively, with no statistically-significant differences observed.

Nominal Conc. ($\mu\text{g ai/L}$) ^(a)	Survival						
	At Hatch ^(b)		At End of Swim-up		At End of Exposure (Day 61)		
	No.	%	No.	%	No.	% of swim-up	% of egg inserted
Negative control	94	94	86	91	77	90	77
3	93	93	88	95	81	94 ^(c)	83 ^(c)
6	94	94	87	93	85	98	85
12	95	95	90	95	79	90 ^(c)	81 ^(c)
24	97	97	90	93	83	93 ^(c)	84 ^(c)
48	96	96	91	95	83	92 ^(c)	84 ^(c)

^(a) Reviewer-calculated TWA concentrations were <1 (<LOQ, control), 3.1, 6.1, 12, 23, and 49 $\mu\text{g ai/L}$ for the F₂ generation exposure period (refer to Appendix II for copy of Excel worksheet).

^(b) 100 initial embryos per level.

^(c) Fish that jumped out of the vessels were considered to be "not at risk".

Time to hatch, time to swim-up, and clinical signs of toxicity: No treatment-related effect on the time to hatch or time to swim-up was indicated. Hatching occurred from Days 2 through 5, and swim-up occurred from Days 3 to 6. In addition, no substance-related signs of toxicity were observed following hatch.

Growth: At 4 weeks post-hatch (Day 35), statistically-significant increases ($p \leq 0.01$) in body length were indicated at the 3 and 48 $\mu\text{g ai/L}$ levels (lowest and highest) compared to the control. Since the deviations were slight (6 and 8%, respectively) and represented an improvement in the endpoint, they were considered incidental to treatment. At 8 weeks post-hatch (Day 61), statistically-significant decreases ($p \leq 0.01$) in body length were observed at the 24 (-6%) and 48 $\mu\text{g ai/L}$ (-5%) levels compared to the control. A statistically-significant reduction in body weight (-19%; $p \leq 0.01$) was also observed at the 24 $\mu\text{g ai/L}$ level compared to the control on Day 61, though the difference at the 48 $\mu\text{g ai/L}$ level (-6%) was not statistically significant. The study author noted that the growth of the 48- $\mu\text{g ai/L}$ group was faster up until Day 35 than that of the control group, and that the growth from Days 35 to 61 at this level was therefore more markedly decreased than the comparison of body weight and length at sacrifice suggest. Thus, this would at least partly explain the inconclusive concentration-effect-relationship for growth of the F_2 generation.

Nominal Conc. ($\mu\text{g ai/L}$)	Body Length: Mean \pm SD (cm)		Body Weight: Mean \pm SD (g)
	Day 35	Day 61	
Negative control	2.47 \pm 0.272	4.5 \pm 0.37	0.941 \pm 0.234
3	2.62 \pm 0.237**	4.4 \pm 0.34	0.918 \pm 0.235
6	2.55 \pm 0.224	4.4 \pm 0.30	0.859 \pm 0.200
12	2.53 \pm 0.241	4.4 \pm 0.30	0.869 \pm 0.200
24	2.48 \pm 0.264	4.2 \pm 0.29**	0.764 \pm 0.184**
48	2.66 \pm 0.216**	4.3 \pm 0.28**	0.886 \pm 0.209

^(a) Reviewer-calculated TWA concentrations were <1 ($<\text{LOQ}$, control), 3.1, 6.1, 12, 23, and 49 $\mu\text{g ai/L}$ for the F_2 generation exposure period (refer to Appendix II for copy of Excel worksheet).

** Statistically-significant difference from the control at $p \leq 0.01$.

Statistical Results:

Statistical Method (s): The 20 data endpoints statistically assessed are summarized in the following table. Nominal concentrations were used in the calculations.

For survival data (F_1 and F_2 generations), a pair-wise comparison of each treatment group with the control group was carried out via Fisher's exact test (one-sided). To consider the variability

between the aquaria, all survival data (for which data was available for at least four aquaria) were analyzed via the Wilcoxon Test (one-sided). The statistical unit was the aquarium.

For the reproductive endpoints, a pair-wise comparison of each treatment group with the control group was carried out via the Wilcoxon Test. The fertility rate was calculated as mean fertility rate per clutch. For the number of eggs per day and the fertility rate, the test was performed one-sided, and for the number of clutches per day, the test was performed two-sided.

Growth data were statistically evaluated using Dunnett's test for a simultaneous comparison of several dose groups with the control group (two-sided).

Biological Endpoint	NOAEC ($\mu\text{g ai/L}$)	LOAEC ($\mu\text{g ai/L}$)
F ₁ survival from start to hatch (to Day 6)	48	>48
F ₁ survival from hatch to swim-up	48	>48
F ₁ survival from swim-up to reduction (to Day 36)	48	>48
F ₁ survival from reduction to reproduction (to Day 68)	48	>48
F ₁ survival from reproduction to sacrifice (to Day 116/117)	48	>48
F ₁ length at 4 weeks post-hatch	48	>48
F ₁ length at 8 weeks post-hatch	48	>48
F ₁ male length at sacrifice of pairs	48	>48
F ₁ female length at sacrifice of pairs	48	>48
F ₁ male weight at sacrifice of pairs	48	>48
F ₁ female weight at sacrifice of pairs	48	>48
F ₁ eggs/female/day	48	>48
F ₁ clutches/female/day	48	>48
F ₁ % fertility	48	>48

Biological Endpoint	NOAEC ($\mu\text{g ai/L}$)	LOAEC ($\mu\text{g ai/L}$)
F ₂ survival from start to hatch	48	>48
F ₂ survival from hatch to swim-up	48	>48
F ₂ survival from swim-up to sacrifice (at 8 weeks)	48	>48
F ₂ 4-week length	48	>48
F ₂ 8-week length	12	24
F ₂ 8-week weight	12	24

NOAEC: 12 $\mu\text{g ai/L}$ LOAEC: 24 $\mu\text{g ai/L}$ **14. REVIEWER'S STATISTICAL RESULTS:**

Statistical Method: For all endpoints where treatment elicited a reduction relative to the control, the data were analyzed to determine if they followed the assumptions of normality (Chi-square and Shapiro-Wilks tests) and homogeneity of variances (Hartley and Bartlett's tests). For most endpoints the assumptions were met and the NOAEC and LOAEC values were determined using ANOVA, followed by Dunnett's or Bonferroni's t-test and/or William's test (dose-dependent responses). These analyses were conducted using Toxstat statistical software.

Biological Endpoint	NOAEC ($\mu\text{g ai/L}$)	LOAEC ($\mu\text{g ai/L}$)
F ₁ survival from start to hatch (to Day 6)	49	>49
F ₁ survival from hatch to swim-up	49	>49
F ₁ survival from swim-up to reduction (to Day 36)	49	>49
F ₁ survival from reduction to reproduction (to Day 68)	49	>49
F ₁ survival from reproduction to sacrifice (to Day 116/117)	43	>43
F ₁ length at 4 weeks post-hatch	49	>49
F ₁ length at 8 weeks post-hatch	49	>49
F ₁ male length at sacrifice of pairs	43	>43
F ₁ female length at sacrifice of pairs	43	>43
F ₁ male weight at sacrifice of pairs	43	>43

Biological Endpoint	NOAEC (µg ai/L)	LOAEC (µg ai/L)
F ₁ female weight at sacrifice of pairs	43	>43
F ₁ eggs/female/day	43	>43
F ₁ clutches/female/day	43	>43
F ₁ % fertility	43	>43
F ₂ survival from start to hatch	49	>49
F ₂ survival from hatch to swim-up	49	>49
F ₂ survival from swim-up to sacrifice (at 8 weeks)	49	>49
F ₂ 4-week length	49	>49
F ₂ 8-week length	12	23
F ₂ 8-week weight	12	23

NOAEC: 12 µg ai/L (TWA)

LOAEC: 23 µg ai/L (TWA)

Most sensitive endpoint(s): F₂-generation growth at 8 weeks post-hatch

Comments: The reviewer's analysis confirmed that there were significant adverse effects of treatment on F₂ generation growth endpoints.

15. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'. The reviewer reports NOAEC and LOAEC values based on the TWA concentrations.

TWA concentrations were reviewer-calculated (refer to associated Excel worksheet in Appendix II). As TWA concentrations are more indicative of actual concentration levels, they were reported in the Statistical Verification and Conclusions sections of the DER. TWA concentrations were calculated using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C TWA is the time-weighted average concentration,

C_j is the concentration measured at time interval j ($j = 0, 1, 2, \dots, n$).

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., $t_0 = 0$ hours (test initiation), $t_1 = 24$ hours, $t_2 = 96$ hours).

The study author noted that both validity requirements delineated in OPPTS 850.1400 guidance (pertaining to early life-stage toxicity tests) were fulfilled in both the F_1 and F_2 generations. Specifically, hatching success of the F_1 - and F_2 -generation controls was 84% and 94%, respectively, fulfilling the minimum requirement of $\geq 66\%$, and post-hatch survival of the F_1 and F_2 -generation controls was 75% (30 days post-hatch) and 77% (60-days post-hatch), fulfilling the minimum requirement of 70% (at ca. 28 days post-hatch).

The test substance was a homogenous white solid identified as BAS 595 F (Triticonazole) from Batch No. COD-00601, and was stored at room temperature. The CAS No. for triticonazole is 131983-72-7; the IUPAC name is *(RS)-(E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol*; and the CAS name is *(5E)-5-[(4-chlorophenyl)methylene]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol*. The reported water solubility was 8.4 mg/L at 20°C, and the $\log p_{ow}$ was 3.29.

In-life dates were July 5 to December 30, 2007.

15. REFERENCES:

- Siegel, S. 1956. Non-parametric statistics for behavioral sciences. McGraw-Hill New York.
- Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Statist. Assoc.* 50:1096-1121.
- Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20:482-491.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.216	11.616	18.336	11.616	3.216
OBSERVED	2	12	16	17	1

Calculated Chi-Square goodness of fit test statistic = 4.7925
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.545

W = 0.919

Critical W (P = 0.05) (n = 48) = 0.947

Critical W (P = 0.01) (n = 48) = 0.929

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and
 should not be performed.

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 2.62
 Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7
 Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

DP Barcode: 361785

MRID No.: 47622101

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

clutch per female per day
File: 2101c Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.12
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

clutch per female per day
File: 2101c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.052	0.010	0.769
Within (Error)	42	0.545	0.013	
Total	47	0.596		

Critical F value = 2.45 (0.05, 5, 40)
Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

clutch per female per day
File: 2101c Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
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DP Barcode: 361785

MRID No.: 47622101

1	control	0.225	0.225	
2	3	0.150	0.150	1.318
3	6	0.175	0.175	0.875
4	12	0.175	0.175	0.877
5	24	0.133	0.133	1.607
6	48	0.217	0.217	0.147

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	8			
2	3	8	0.132	58.5	0.075
3	6	8	0.132	58.5	0.050
4	12	8	0.132	58.5	0.050
5	24	8	0.132	58.5	0.092
6	48	8	0.132	58.5	0.008

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	0.225	0.225	0.225
2	3	8	0.150	0.150	0.170
3	6	8	0.175	0.175	0.170
4	12	8	0.175	0.175	0.170
5	24	8	0.133	0.133	0.170
6	48	8	0.217	0.217	0.170

clutch per female per day

File: 2101c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	0.225				

DP Barcode: 361785

MRID No.: 47622101

3	0.170	0.966	1.68	k= 1, v=42
6	0.170	0.966	1.76	k= 2, v=42
12	0.170	0.966	1.79	k= 3, v=42
24	0.170	0.966	1.80	k= 4, v=42
48	0.170	0.966	1.80	k= 5, v=42

s = 0.114

Note: df used for table values are approximate when $v > 20$.

eggs per female per day

File: 2101e Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.216	11.616	18.336	11.616	3.216
OBSERVED	0	16	20	8	4

Calculated Chi-Square goodness of fit test statistic = 6.3383

Table Chi-Square value ($\alpha = 0.01$) = 13.277

Data PASS normality test. Continue analysis.

eggs per female per day

File: 2101e Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 9201.622

W = 0.919

Critical W ($P = 0.05$) ($n = 48$) = 0.947

Critical W ($P = 0.01$) ($n = 48$) = 0.929

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

eggs per female per day

File: 2101e Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

DP Barcode: 361785

MRID No.: 47622101

Calculated H statistic (max Var/min Var) = 3.61
Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)
Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

eggs per female per day
File: 2101e Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 5.21
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

eggs per female per day
File: 2101e Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	86.497	17.299	0.079
Within (Error)	42	9201.622	219.086	
Total	47	9288.119		

Critical F value = 2.45 (0.05, 5, 40)
Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

DP Barcode: 361785

MRID No.: 47622101

eggs per female per day
File: 2101e Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	17.900	17.900		
2	3	14.888	14.888	0.407	
3	6	16.375	16.375	0.206	
4	12	17.250	17.250	0.088	
5	24	17.400	17.400	0.068	
6	48	14.262	14.262	0.492	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

eggs per female per day
File: 2101e Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	8			
2	3	8	17.096	95.5	3.013
3	6	8	17.096	95.5	1.525
4	12	8	17.096	95.5	0.650
5	24	8	17.096	95.5	0.500
6	48	8	17.096	95.5	3.638

eggs per female per day
File: 2101e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2					
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	17.900	17.900	17.900
2	3	8	14.888	14.888	16.478
3	6	8	16.375	16.375	16.478
4	12	8	17.250	17.250	16.478
5	24	8	17.400	17.400	16.478
6	48	8	14.262	14.262	14.262

DP Barcode: 361785

MRID No.: 47622101

eggs per female per day

File: 2101e Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CAIC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	17.900				
3	16.478	0.192		1.68	k= 1, v=42
6	16.478	0.192		1.76	k= 2, v=42
12	16.478	0.192		1.79	k= 3, v=42
24	16.478	0.192		1.80	k= 4, v=42
48	14.262	0.492		1.80	k= 5, v=42

s = 14.802

Note: df used for table values are approximate when v > 20.

% fert

File: 2101f Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.680	9.680	15.280	9.680	2.680
OBSERVED	4	7	17	12	0

Calculated Chi-Square goodness of fit test statistic = 4.8218
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis..

% fert

File: 2101f Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 38.302

W = 0.959

Critical W (P = 0.05) (n = 40) = 0.940

Critical W (P = 0.01) (n = 40) = 0.919

Data PASS normality test at P=0.01 level. Continue analysis.

% fert
File: 2101f Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 11.40
Closest, conservative, Table H statistic = 25.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 6
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 5.67
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

% fert
File: 2101f Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 10.90
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 5.67
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

% fert
File: 2101f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7.662	1.532	1.359
Within (Error)	34	38.302	1.127	

DP Barcode: 361785

MRID No.: 47622101

Total 39 45.964

Critical F value = 2.53 (0.05,5,30)
Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

% fert
File: 2101f Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	98.625	98.625		
2	3	98.440	98.440	0.306	
3	6	98.217	98.217	0.712	
4	12	99.033	99.033	-0.712	
5	24	99.429	99.429	-1.463	
6	48	98.263	98.263	0.683	

Bonferroni T table value = 2.44 (1 Tailed Value, $P=0.05$, $df=34,5$)

% fert
File: 2101f Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	8			
2	3	5	1.478	1.5	0.185
3	6	6	1.400	1.4	0.408
4	12	6	1.400	1.4	-0.408
5	24	7	1.342	1.4	-0.804
6	48	8	1.296	1.3	0.362

% fert
File: 2101f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2					
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	98.625	98.625	98.772
2	3	5	98.440	98.440	98.772
3	6	6	98.217	98.217	98.772

DP Barcode: 361785

MRID No.: 47622101

4	12	6	99.033	99.033	98.772
5	24	7	99.429	99.429	98.772
6	48	8	98.263	98.263	98.263

% fert
File: 2101f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	98.772				
3	98.772	0.243		1.70	k= 1, v=34
6	98.772	0.256		1.78	k= 2, v=34
12	98.772	0.256		1.80	k= 3, v=34
24	98.772	0.267		1.81	k= 4, v=34
48	98.263	0.683		1.82	k= 5, v=34

s = 1.061

Note: df used for table values are approximate when v > 20.

% hatched (end of swim-up
File: 2101fert Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	6	11	7	0

Calculated Chi-Square goodness of fit test statistic = 3.8331
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

% hatched (end of swim-up
File: 2101fert Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 246.750

W = 0.967

DP Barcode: 361785

MRID No.: 47622101

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

% hatched (end of swim-up

File: 2101fert Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 8.07

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	6,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	6,	df (# avg reps-1) =	3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

% hatched (end of swim-up

File: 2101fert Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 4.13

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

% hatched (end of swim-up

File: 2101fert Transform: NO TRANSFORMATION

ANOVA TABLE

DP Barcode: 361785

MRID No.: 47622101

SOURCE	DF	SS	MS	F
Between	5	107.208	21.442	1.564
Within (Error)	18	246.750	13.708	
Total	23	353.958		

Critical F value = 2.77 (0.05,5,18)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

% hatched (end of swim-up)

File: 2101fert

Transform: NO TRANSFORMATION

DUNNETTS TEST

TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	91.500	91.500		
2	3	94.250	94.250	-1.050	
3	6	87.250	87.250	1.623	
4	12	90.250	90.250	0.477	
5	24	89.750	89.750	0.668	
6	48	89.750	89.750	0.668	

Dunnett table value = 2.41 (1 Tailed Value, $P=0.05$, $df=18,5$)

% hatched (end of swim-up)

File: 2101fert

Transform: NO TRANSFORMATION

DUNNETTS TEST

TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3	4	6.309	6.9	-2.750
3	6	4	6.309	6.9	4.250
4	12	4	6.309	6.9	1.250
5	24	4	6.309	6.9	1.750
6	48	4	6.309	6.9	1.750

% hatched (end of swim-up)

File: 2101fert

Transform: NO TRANSFORMATION

WILLIAMS TEST

(Isotonic regression model)

TABLE 1 OF 2

DP Barcode: 361785

MRID No.: 47622101

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	91.500	91.500	92.875
2	3	4	94.250	94.250	92.875
3	6	4	87.250	87.250	89.250
4	12	4	90.250	90.250	89.250
5	24	4	89.750	89.750	89.250
6	48	4	89.750	89.750	89.250

% hatched (end of swim-up

File: 2101fert

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	92.875				
3	92.875	0.525		1.73	k= 1, v=18
6	89.250	0.859		1.82	k= 2, v=18
12	89.250	0.859		1.85	k= 3, v=18
24	89.250	0.859		1.86	k= 4, v=18
48	89.250	0.859		1.87	k= 5, v=18

s = 3.702

Note: df used for table values are approximate when v > 20.

% of swim-up (red to 15)

File: 2101su

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	9	6	9	0

Calculated Chi-Square goodness of fit test statistic = 7.8193

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

% of swim-up (red to 15)

File: 2101su

Transform: NO TRANSFORMATION

DP Barcode: 361785

MRID No.: 47622101

Shapiro Wilks test for normality

D = 218.250

W = 0.927

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.33

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	6,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	6,	df (# avg reps-1) =	3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.27

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

DP Barcode: 361785

MRID No.: 47622101

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	32.708	6.542	0.540
Within (Error)	18	218.250	12.125	
Total	23	250.958		

Critical F value = 2.77 (0.05,5,18)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

DUNNETTS TEST

TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	97.250	97.250		
2	3	95.000	95.000	0.914	
3	6	98.750	98.750	-0.609	
4	12	96.250	96.250	0.406	
5	24	97.500	97.500	-0.102	
6	48	97.500	97.500	-0.102	

Dunnett table value = 2.41 (1 Tailed Value, $P=0.05$, $df=18,5$)

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

DUNNETTS TEST

TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3	4	5.934	6.1	2.250
3	6	4	5.934	6.1	-1.500
4	12	4	5.934	6.1	1.000
5	24	4	5.934	6.1	-0.250
6	48	4	5.934	6.1	-0.250

DP Barcode: 361785

MRID No.: 47622101

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	97.250	97.250	96.125
2		3	95.000	95.000	96.125
3		6	98.750	98.750	97.500
4		12	96.250	96.250	97.500
5		24	97.500	97.500	97.500
6		48	97.500	97.500	97.500

% of swim-up (red to 15)

File: 2101su Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	96.125				
3	96.125	0.457		1.73	k= 1, v=18
6	97.500	0.102		1.82	k= 2, v=18
12	97.500	0.102		1.85	k= 3, v=18
24	97.500	0.102		1.86	k= 4, v=18
48	97.500	0.102		1.87	k= 5, v=18

s = 3.482

Note: df used for table values are approximate when v > 20.

% of reduced

File: 2101sr Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	100.000	100.000	58.000
2		100.000	100.000	58.000
3		98.250	98.250	46.500
4		95.000	95.000	33.000
5		98.250	98.250	46.500
6		100.000	100.000	58.000

Calculated H Value = 6.018

Critical H Value Table = 11.070

DP Barcode: 361785

MRID No.: 47622101

Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

% of reduced

File: 2101sr

Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP					
				0	0	0	0	0	0
4	12	95.000	95.000	\					
3	6	98.250	98.250	.	\				
5	24	98.250	98.250	.	.	\			
2	3	100.000	100.000	.	.	.	\		
1	control	100.000	100.000	\	
6	48	100.000	100.000	\

* = significant difference (p=0.05)

Table q value (0.05,6) = 2.936

. = no significant difference

SE = 3.240

body weight males sacrifice

File: 2101wm

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.305	0.061	0.137
Within (Error)	42	18.679	0.445	
Total	47	18.985		

Critical F value = 2.45 (0.05,5,40)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

body weight males sacrifice

File: 2101wm

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4.455	4.455		
2	3	4.423	4.423	0.097	
3	6	4.440	4.440	0.045	

DP Barcode: 361785

MRID No.: 47622101

4	12	4.365	4.365	0.270
5	24	4.221	4.221	0.701
6	48	4.426	4.426	0.086

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	control	8				
2	3	8	0.770	17.3	0.033	
3	6	8	0.770	17.3	0.015	
4	12	8	0.770	17.3	0.090	
5	24	8	0.770	17.3	0.234	
6	48	8	0.770	17.3	0.029	

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	4.455	4.455	4.455
2	3	8	4.423	4.423	4.431
3	6	8	4.440	4.440	4.431
4	12	8	4.365	4.365	4.365
5	24	8	4.221	4.221	4.324
6	48	8	4.426	4.426	4.324

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	4.455				
3	4.431	0.071		1.68	k= 1, v=42
6	4.431	0.071		1.76	k= 2, v=42
12	4.365	0.270		1.79	k= 3, v=42
24	4.324	0.394		1.80	k= 4, v=42

DP Barcode: 361785

MRID No.: 47622101

48 4.324 0.394 1.80 k= 5, v=42

s = 0.667

Note: df used for table values are approximate when v > 20.

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.216	11.616	18.336	11.616	3.216
OBSERVED	4	11	18	12	3

Calculated Chi-Square goodness of fit test statistic = 0.2571

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 18.679

W = 0.984

Critical W (P = 0.05) (n = 48) = 0.947

Critical W (P = 0.01) (n = 48) = 0.929

Data PASS normality test at P=0.01 level. Continue analysis.

body weight males sacrifice

File: 2101wm Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.98

Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 7

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 7.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

body weight males sacrifice
File: 2101wm Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 6.98
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

body weight female sacrifice
File: 2101wf Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.082	11.132	17.572	11.132	3.082
OBSERVED	2	13	19	9	3

Calculated Chi-Square goodness of fit test statistic = 1.2199
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

body weight female sacrifice
File: 2101wf Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

DP Barcode: 361785

MRID No.: 47622101

D = 9.836

W = 0.845

Critical W (P = 0.05) (n = 46) = 0.945

Critical W (P = 0.01) (n = 46) = 0.927

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

body weight female sacrifice

File: 2101wf Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 11.26

Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==>	R (# groups) = 6,	df (# reps-1) = 7
Actual values ==>	R (# groups) = 6,	df (# avg reps-1) = 6.67
		(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

body weight female sacrifice

File: 2101wf Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 16.06

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 6.67

Used for Chi-square table value ==> df (#groups-1) = 5

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

DP Barcode: 361785

MRID No.: 47622101

body weight female sacrifice
File: 2101wf Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.657	0.131	0.533
Within (Error)	40	9.836	0.246	
Total	45	10.493		

Critical F value = 2.45 (0.05,5,40)
Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

body weight female sacrifice
File: 2101wf Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2.095	2.095		
2	3	2.334	2.334	-0.932	
3	6	2.237	2.237	-0.575	
4	12	2.320	2.320	-0.877	
5	24	2.095	2.095	0.000	
6	48	2.018	2.018	0.313	

Bonferroni T table value = 2.42 (1 Tailed Value, $P=0.05$, $df=40, 5$)

body weight female sacrifice
File: 2101wf Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	8			
2	3	7	0.622	29.7	-0.239
3	6	8	0.601	28.7	-0.142
4	12	7	0.622	29.7	-0.225
5	24	8	0.601	28.7	0.000
6	48	8	0.601	28.7	0.077

DP Barcode: 361785

MRID No.: 47622101

body weight female sacrifice

File: 2101wf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	2.095	2.095	2.241
2	3	7	2.334	2.334	2.241
3	6	8	2.237	2.237	2.241
4	12	7	2.320	2.320	2.241
5	24	8	2.095	2.095	2.095
6	48	8	2.018	2.018	2.018

body weight female sacrifice

File: 2101wf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	2.241				
3	2.241	0.570		1.68	k= 1, v=40
6	2.241	0.590		1.76	k= 2, v=40
12	2.241	0.570		1.79	k= 3, v=40
24	2.095	0.000		1.80	k= 4, v=40
48	2.018	0.313		1.80	k= 5, v=40

s = 0.496

Note: df used for table values are approximate when v > 20.

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.283	11.858	18.718	11.858	3.283
OBSERVED	2	12	22	9	4

Calculated Chi-Square goodness of fit test statistic = 1.9240

Table Chi-Square value (alpha = 0.01) = 13.277

DP Barcode: 361785

MRID No.: 47622101

Data PASS normality test. Continue analysis.

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 4.430

W = 0.979

Critical W (P = 0.05) (n = 49) = 0.947

Critical W (P = 0.01) (n = 49) = 0.929

Data PASS normality test at P=0.01 level. Continue analysis.

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 6.10

Closest, conservative, Table H statistic = 18.4 (alpha = 0.01)

Used for Table H ==>	R (# groups) = 6,	df (# reps-1) = 7
Actual values ==>	R (# groups) = 6,	df (# avg reps-1) = 7.17
		(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 5.98

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 7.17

DP Barcode: 361785

MRID No.: 47622101

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.552	0.110	1.068
Within (Error)	43	4.430	0.103	
Total	48	4.982		

Critical F value = 2.45 (0.05,5,40)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	6.700	6.700		
2	3	6.856	6.856	-0.997	
3	6	6.613	6.613	0.545	
4	12	6.925	6.925	-1.402	
5	24	6.725	6.725	-0.156	
6	48	6.688	6.688	0.078	

Bonferroni T table value = 2.42 (1 Tailed Value, P=0.05, df=40,5)

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
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DP Barcode: 361785

MRID No.: 47622101

1	control	8			
2	3	9	0.378	5.6	-0.156
3	6	8	0.389	5.8	0.088
4	12	8	0.389	5.8	-0.225
5	24	8	0.389	5.8	-0.025
6	48	8	0.389	5.8	0.013

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	8	6.700	6.700	6.782
2	3	9	6.856	6.856	6.782
3	6	8	6.613	6.613	6.769
4	12	8	6.925	6.925	6.769
5	24	8	6.725	6.725	6.725
6	48	8	6.688	6.688	6.688

length male sacrifice

File: 2101ml Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	6.782				
3	6.782	0.528		1.68	k= 1, v=43
6	6.769	0.428		1.76	k= 2, v=43
12	6.769	0.428		1.79	k= 3, v=43
24	6.725	0.156		1.80	k= 4, v=43
48	6.688	0.078		1.80	k= 5, v=43

s = 0.321

Note: df used for table values are approximate when v > 20.

F2 body length

File: 2101f2l Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
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DP Barcode: 361785

MRID No.: 47622101

EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	10	5	9	0

Calculated Chi-Square goodness of fit test statistic = 9.8908
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

F2 body length
File: 2101f21 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.191

W = 0.980

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

F2 body length
File: 2101f21 Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 64.40
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	6,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	6,	df (# avg reps-1) =	3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

F2 body length
File: 2101f21 Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

DP Barcode: 361785

MRID No.: 47622101

Calculated B statistic = 10.39
 Table Chi-square value = 15.09 (alpha = 0.01)
 Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
 Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

F2 body length
 File: 2101f21

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.119	0.024	2.182
Within (Error)	18	0.191	0.011	
Total	23	0.310		

Critical F value = 2.77 (0.05,5,18)

Since F < Critical F FAIL TO REJECT Ho: All groups equal

F2 body length
 File: 2101f21

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2.470	2.470		
2	3	2.625	2.625	-2.090	
3	6	2.548	2.548	-1.045	
4	12	2.530	2.530	-0.809	
5	24	2.478	2.478	-0.101	
6	48	2.660	2.660	-2.562	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

F2 body length
 File: 2101f21

Transform: NO TRANSFORMATION

DP Barcode: 361785

MRID No.: 47622101

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	control	4				
2	3	4	0.179	7.2	-0.155	
3	6	4	0.179	7.2	-0.077	
4	12	4	0.179	7.2	-0.060	
5	24	4	0.179	7.2	-0.007	
6	48	4	0.179	7.2	-0.190	

F2 body length

File: 2101f21

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	2.470	2.470	2.470
2	3	4	2.625	2.625	2.545
3	6	4	2.548	2.548	2.545
4	12	4	2.530	2.530	2.545
5	24	4	2.478	2.478	2.545
6	48	4	2.660	2.660	2.660

F2 body length

File: 2101f21

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	2.470				
3	2.545	1.030		1.73	k= 1, v=18
6	2.545	1.030		1.82	k= 2, v=18
12	2.545	1.030		1.85	k= 3, v=18
24	2.545	1.030		1.86	k= 4, v=18
48	2.660	2.609	*	1.87	k= 5, v=18

s = 0.103

Note: df used for table values are approximate when v > 20.

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	8	9	7	0

Calculated Chi-Square goodness of fit test statistic = 4.2910

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

F2 weight

File: 2101f2w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.118

W = 0.974

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

F2 weight

File: 2101f2w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 12.90

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) = 6,	df (# reps-1) = 3
Actual values ==>	R (# groups) = 6,	df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

DP Barcode: 361785

MRID No.: 47622101

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 5.26

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.077	0.015	2.143
Within (Error)	18	0.118	0.007	
Total	23	0.195		

Critical F value = 2.77 (0.05,5,18)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

DUNNETTS TEST

TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.942	0.942		
2	3	0.920	0.920	0.372	
3	6	0.861	0.861	1.369	
4	12	0.869	0.869	1.242	
5	24	0.764	0.764	3.017	*
6	48	0.885	0.885	0.968	

DP Barcode: 361785

MRID No.: 47622101

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	control	4				
2	3	4	0.143	15.1	0.022	
3	6	4	0.143	15.1	0.081	
4	12	4	0.143	15.1	0.074	
5	24	4	0.143	15.1	0.179	
6	48	4	0.143	15.1	0.057	

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	0.942	0.942	0.942
2	3	4	0.920	0.920	0.920
3	6	4	0.861	0.861	0.865
4	12	4	0.869	0.869	0.865
5	24	4	0.764	0.764	0.824
6	48	4	0.885	0.885	0.824

F2 weight

File: 2101f2w

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	0.942				
3	0.920	0.384		1.73	k= 1, v=18
6	0.865	1.348		1.82	k= 2, v=18
12	0.865	1.348		1.85	k= 3, v=18
24	0.824	2.057	*	1.86	k= 4, v=18
48	0.824	2.057	*	1.87	k= 5, v=18

s = 0.081

Note: df used for table values are approximate when v > 20.

DP Barcode: 361785

MRID No.: 47622101

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	9	7	8	0

Calculated Chi-Square goodness of fit test statistic = 6.3102

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.283

W = 0.980

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 10.75

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

DP Barcode: 361785

MRID No.: 47622101

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

F2 length on d 61
File: 2101f212 Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 6.64
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

F2 length on d 61
File: 2101f212 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.224	0.045	2.813
Within (Error)	18	0.283	0.016	
Total	23	0.506		

Critical F value = 2.77 (0.05,5,18)
Since F > Critical F REJECT Ho: All groups equal

F2 length on d 61
File: 2101f212 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4.525	4.525		

DP Barcode: 361785

MRID No.: 47622101

2	3	4.425	4.425	1.118
3	6	4.400	4.400	1.398
4	12	4.450	4.450	0.839
5	24	4.250	4.250	3.075 *
6	48	4.275	4.275	2.795 *

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	3	4	0.216	4.8	0.100
3	6	4	0.216	4.8	0.125
4	12	4	0.216	4.8	0.075
5	24	4	0.216	4.8	0.275
6	48	4	0.216	4.8	0.250

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	4.525	4.525	4.525
2	3	4	4.425	4.425	4.425
3	6	4	4.400	4.400	4.425
4	12	4	4.450	4.450	4.425
5	24	4	4.250	4.250	4.263
6	48	4	4.275	4.275	4.263

F2 length on d 61

File: 2101f212

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	4.525				
3	4.425	1.129		1.73	k= 1, v=18
6	4.425	1.129		1.82	k= 2, v=18

DP Barcode: 361785

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12	4.425	1.129		1.85	k= 3, v=18
24	4.263	2.964	*	1.86	k= 4, v=18
48	4.263	2.964	*	1.87	k= 5, v=18

s = 0.125

Note: df used for table values are approximate when $v > 20$.

**APPENDIX II. COPY OF REVIEWER'S TIME-WEIGHTED AVERAGE (TWA)
CALCULATIONS USING EXCEL SOFTWARE:**

F1 Generation Prior to Spawning
Phase

Nominal Concentration (ug/L)	Time (Day)	Measured Concentration (ug ai/L)	TWA (ug ai/L)
3	0	2.7	
	6	2.5	
	13	3.2	
	20	3.3	
	28	3.2	
	34	3.2	
	41	2.7	
	48	3.9	
	49	2.9	
	55	3	
	62	3.4	
	69	2.8	
	76	2.8	
	83	3.2	
	91	3.8	
	92	2.8	
		TWA High-low ratio	3.1 1.5
6	0	6.4	
	6	5.3	
	13	6.4	
	20	8.4	
	21	7.2	
	28	7.2	
	34	5.9	
	41	4.8	
	48	7.7	
	49	5.2	
	55	5.4	
	62	5.1	
	69	6.3	
	76	5.1	
	83	6.2	
	91	6.3	
		TWA High-low ratio	6.1 1.8

DP Barcode: 361785

MRID No.: 47622101

12	0	9.7		
	6	7.8		
	7	9.6		
	13	13		
	20	15.9		
	21	13.8		
	28	12.8		
	34	13.5		
	41	9.7		
	48	15.1		
	49	11.3		
	55	11.7		
	62	12.8		
	69	12.1		
	76	10		
	83	13.1		
	91	12		
		TWA	12.1	
		High-low ratio	2.0	

24	0	24.4		
	6	21.9		
	13	24.2		
	20	31.2		
	21	27.8		
	28	27.3		
	34	25.5		
	41	21.8		
	48	24.3		
	55	24.9		
	62	25.9		
	69	26.5		
	76	22.3		
	83	33.4		
	84	19.6		
	91	20.7		
		TWA	24.9	
		High-low ratio	1.7	

48	0	53		
	6	41.7		
	13	46.2		
	20	58.3		
	21	52.2		
	28	52.3		
	34	46.6		

DP Barcode: 361785

MRID No.: 47622101

41	38.6
48	55.3
55	55.6
62	48.5
69	50.7
76	43.8
83	47.5
91	52.5

TWA	48.9
High-low ratio	1.5

F1 Generation Spawning Groups

Nominal Concentration (ug/L)	Time (Day)	Measured Concentration (ug ai/L)	TWA (ug ai/L)
3	95	2.4	
	98	2.8	
	104	2.9	
	111	2.8	
	118	2.8	
	125	2.7	
		TWA	2.8
		High-low ratio	1.2
6	95	5.55	
	104	6.05	
	111	5.8	
	118	5.55	
	125	5.85	
		TWA	5.8
		High-low ratio	1.1
12	95	10.05	
	104	11.15	
	111	11.2	
	118	10.9	
	125	11.55	
		TWA	11.0
		High-low ratio	1.1
24	95	19.9	
	104	23.95	
	111	21.7	
	118	21.15	
		59	

DP Barcode: 361785

MRID No.: 47622101

	125	21.2	
		TWA	21.8
		High-low ratio	1.2
48	95	37.45	
	98	40.8	
	104	46.35	
	112	40.7	
	118	42.5	
	125	47.35	
		TWA	43.0
		High-low ratio	1.3

F2 Generation

Nominal Concentration (ug/L)	Time (Day)	Measured Concentration (ug ai/L)	TWA (ug ai/L)
3	116.5	2.2	
	119	1.8	
	125	3.3	
	132	2.2	
	133	3	
	139	3.2	
	146	4.1	
	147	3.2	
	153	2.8	
	160	3.4	
	167	3.3	
	175	3.6	
		TWA	3.1
		High-low ratio	2.3
6	116.5	4	
	119	4.3	
	125	6	
	132	4.8	
	133	5.7	
	139	6.4	
	146	9.6	
	147	6.2	
	153	5.5	
	160	5.8	
	167	6.5	
	175	7.1	

DP Barcode: 361785

MRID No.: 47622101

		TWA	6.1
		High-low ratio	2.4
12	116.5	8.9	
	119	9.5	
	125	11.1	
	132	8.9	
	133	11.1	
	134	11	
	139	13.1	
	146	15.6	
	147	12.2	
	153	10.8	
	160	11.4	
	167	13.2	
	175	14.2	
		TWA	11.8
		High-low ratio	1.8
24	116.5	20.4	
	125	20.1	
	132	16.7	
	133	22.6	
	139	23.2	
	146	26.4	
	153	18.3	
	154	19.2	
	160	24.6	
	167	27.2	
	175	28.7	
		TWA	23.0
		High-low ratio	1.6
48	116.5	37.8	
	119	40.8	
	125	50.1	
	132	43	
	133	58.5	
	139	51.5	
	146	52.9	
	153	37.1	
	154	38.7	
	160	53	
	167	53.3	
	175	56.1	
		TWA	49.2
		High-low ratio	1.6